

Set	Items	Description
S1	989	TRANSLATION(S) TERMINAT? (S) CODON? ?
S2	4123	RESPIRATORY (W) SYNCYTIAL (W) VIRUS
S3	1	S1 AND S2
S4	0	TERMINATION (W) CODOD? ?
S5	1697	TERMINATION (W) CODON? ?
S6	4	S2 AND S5
S7	3	S6 NOT S3
S8	3	RD (unique items)
S9	145652	RECOMINANT OR VECTOR? ? OR COSMID? ? OR PLASMID? ?
S10	91694	MUTAGEN?
S11	178484	RECOMBINANT
S12	291010	S9 OR S11
S13	2	S2 AND S5 AND S12
S14	2592	TERMINATION (S) CODON? ?
S15	716	S14 AND S12
S16	2	S15 AND S2
S17	115	S15 AND S10
S18	93	S17 NOT PY>1996
S19	93	RD (unique items)
S20	121	S10 (S) S14
S21	58	S18 AND S20
S22	77	S10 (S) S5
S23	39	S22 AND S12
S24	31	S23 NOT PY>1996
S25	31	RD (unique items)

25/9/10 (Item 10 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07482419 92098583 PMID: 1757470

Mutagenesis of the 43-kD postsynaptic protein defines domains involved in plasma membrane targeting and AChR clustering.

Phillips WD; Maimone MM; Merlie JP
Department of Molecular Biology and Pharmacology, Washington University
School of Medicine, St. Louis, Missouri 63110.

Journal of cell biology (UNITED STATES) Dec 1991, 115 (6) p1713-23,
ISSN 0021-9525 Journal Code: HMV

Contract/Grant No.: 5-T32-HL07275, HL, NHLBI

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

The postsynaptic membrane of the neuromuscular junction contains a myristoylated 43-kD protein (43k) that is closely associated with the cytoplasmic face of the nicotinic acetylcholine receptor (AChR)-rich plasma membrane. Previously, we described fibroblast cell lines expressing **recombinant** AChRs. Transfection of these cell lines with 43k was necessary and sufficient for reorganization of AChR into discrete 43k-rich plasma membrane domains (Phillips, W. D., C. Kopta, P. Blount, P. D. Gardner, J. H. Steinbach, and J. P. Merlie. 1991. Science (Wash. DC). 251:568-570). Here we demonstrate the utility of this expression system for the study of 43k function by site-directed **mutagenesis**. Substitution of a **termination codon** for Asp254 produced a truncated (28-kD) protein that associated poorly with the cell membrane. The conversion of Gly2 to Ala2, to preclude NH2-terminal myristoylation, reduced the frequency with which 43k formed plasma membrane domains by threefold, but did not eliminate the aggregation of AChRs at these domains. Since both NH2 and COOH-termini seemed important for association of 43k with the plasma membrane, a deletion mutant was constructed in which the codon Gln15 was fused in-frame to Ile255 to create a 19-kD protein. This mutated protein formed 43k-rich plasma membrane domains at wild-type frequency, but the domains failed to aggregate AChRs, suggesting that the central part of the 43k polypeptide may be involved in AChR aggregation. Our results suggest that membrane association and AChR interactions are separable functions of the 43k molecule.

Tags: Animal; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
Descriptors: *Cell Membrane--metabolism--ME; *Muscle Proteins--metabolism--ME; *Neuromuscular Junction--metabolism--ME; *Receptors, Cholinergic--metabolism--ME; Cells, Cultured; Fibroblasts--metabolism--ME; Fluorescent Antibody Technique; Immunoblotting; Muscle Proteins--genetics--GE; Mutagenesis, Site-Directed; Quail; Receptor Aggregation; **Recombinant** Proteins--genetics--GE; **Recombinant** Proteins--metabolism--ME
CAS Registry No.: 0 (Muscle Proteins); 0 (Receptors, Cholinergic); 0 (Recombinant Proteins); 0 (peripheral membrane protein 43K)
Record Date Created: 19920205

25/9/14 (Item 14 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07039124 93172336 PMID: 8437213

The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks.

Chen HS; Kaneko S; Girones R; Anderson RW; Hornbuckle WE; Tennant BC; Cote PJ; Gerin JL; Purcell RH; Miller RH

Hepatitis Viruses Section, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20892.

Journal of virology (UNITED STATES) Mar 1993, 67 (3) p1218-26,

ISSN 0022-538X Journal Code: KCV

Contract/Grant No.: NO1-AI-72623, AI, NIAID; NO1-AI-82698, AI, NIAID

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

All mammalian hepadnaviruses possess a gene, termed X, that encodes a protein capable of transactivating virus gene expression. The X gene overlaps the polymerase and precore genes as well as two newly identified open reading frames (ORFs) termed ORF5 and ORF6. In this investigation, we examined whether ORF5, ORF6, and the X gene were important for the replication of woodchuck hepatitis virus (WHV) in susceptible woodchucks. First, we investigated whether proteins were produced from ORF5 and ORF6 by in vitro translation of appropriate viral transcripts, searched for antibodies against the putative proteins in the sera of animals infected with wild-type virus, and looked for an antisense WHV transcript, necessary for expression of a protein from ORF6, in the livers of acutely or chronically infected woodchucks. All such experiments yielded negative results. Next, we used oligonucleotide-directed **mutagenesis** to introduce **termination codons** into ORF5 and ORF6 at two locations within each ORF. Adult woodchucks in groups of three were transfected with one of the four mutant genomes. All of these woodchucks developed WHV infections that were indistinguishable from those of animals transfected with the wild-type WHV **recombinant**. Polymerase chain reaction amplification and direct DNA sequencing confirmed that reversion of the mutants to a wild-type genotype did not occur. Taken together, these data indicate that ORF5 and ORF6 are not essential for virus replication and are unlikely to represent authentic genes. Finally, we generated five WHV X-gene mutants that either removed the initiation codon for protein synthesis or truncated the carboxyl terminus of the protein by 3, 16, 31, or 52 amino acids. Groups of three adult woodchucks were transfected with one of the five X-gene mutants. Only the mutant that possessed an X gene lacking 3 amino acids from the carboxyl terminus was capable of replication within the 6-month time frame of the experiment. In contrast, all seven woodchucks transfected with wild-type WHV DNA developed markers consistent with viral infection. Thus, it is likely ($P < 0.01$) that the WHV X gene is important for virus replication in the natural host.

Tags: Animal; Comparative Study; Support, U.S. Gov't, P.H.S.

Descriptors: *Genes, Viral--genetics--GE; *Hepatitis B Virus --pathogenicity--PY; *Hepatitis, Viral, Animal--genetics--GE; *Marmota --microbiology--MI; *Viral Proteins--genetics--GE; Amino Acid Sequence; Animals, Newborn; Antibodies, Viral--blood--BL; Biological Markers; Genome, Viral; Hepatitis B Virus--genetics--GE; Hepatitis B Virus--growth and development--GD; Immunoassay; Liver--microbiology--MI; Molecular Sequence Data; Mutagenesis, Site-Directed; Open Reading Frames--genetics--GE; RNA Precursors--genetics--GE; RNA, Antisense--analysis--AN; Time Factors; Transcription, Genetic; Transfection; Translation, Genetic; Viral Proteins --biosynthesis--BI; Virulence

CAS Registry No.: 0 (Antibodies, Viral); 0 (Biological Markers); 0 (RNA Precursors); 0 (RNA, Antisense); 0 (Viral Proteins)

Record Date Created: 19930323

25/9/22 (Item 22 from file: 155)

DIALOG(R) File 155:MEDLINE(R)

06734616 91082434 PMID: 1984659

Efficient in vitro translation and processing of the rubella virus structural proteins in the presence of microsomes.

Marr LD; Sanchez A; Frey TK

Department of Biology, Georgia State University, Atlanta 30302-4010.

Virology (UNITED STATES) Jan 1991, 180 (1) p400-5, ISSN 0042-6822

Journal Code: XEA

Contract/Grant No.: AI-00923, AI, NIAID; AI-21389, AI, NIAID
 Languages: ENGLISH
 Document type: Journal Article
 Record type: Completed
 Subfile: INDEX MEDICUS

In the structural protein open reading frame (SP-ORF) of rubella virus (RUB), the sequences for the three virion proteins occur in the order NH2-C-E2-E1-COOH with hydrophobic, consensus signal sequences preceding the amino termini for each of the two membrane proteins (T. K. Frey and L. D. Marr, 1988 Gene 62, 85-100). In vitro translation in the presence of microsomes of RNA transcripts from a **plasmid** containing the SP-ORF resulted in production and accurate processing of the three structural proteins. Since in the absence of microsomes the 110-kDa precursor of these proteins is produced, this finding indicated that the cleavage events in processing of the precursor were mediated by signalase. To study the C-E2 processing event, a DNA construct was made which contained the sequences for E2 beginning at the NH2 terminus of the hydrophobic consensus signal and extending through to the NH2 terminus of E1. In vitro translation of transcripts from this construct in the presence of microsomes resulted in accurate processing of E2 confirming that the hydrophobic sequence was a signal sequence and demonstrating it could function externally as well as internally within the 110-kDa precursor. To determine if the E2 signal was maintained on C after cleavage of the precursor by signalase, the SP-ORF **plasmid** was **mutagenized** to place translation **termination codons** at either the NH2 or COOH side of the E2 signal sequence such that C protein lacking or containing the E2 signal would be produced. As expected, the C-minus-signal protein migrated more rapidly in polyacrylamide gels than did the C-plus-signal protein. C translated from the SP-ORF construct as well as authentic C from infected cells comigrated with the C-plus-signal protein, indicating that the E2 signal was not removed. In a corollary study, it was found that RUB C protein was phosphorylated in vivo, although the percentage of the protein phosphorylated was not determined.

Tags: Animal; In Vitro; Support, U.S. Gov't, P.H.S.

Descriptors: *Microsomes; *Protein Processing, Post-Translational; *Rubella Virus--genetics--GE; *Translation, Genetic--genetics--GE; *Viral Structural Proteins--biosynthesis--BI; Antibodies, Monoclonal; Base Sequence; Dogs; Electrophoresis, Polyacrylamide Gel; Molecular Sequence Data; Mutagenesis; Open Reading Frames; Phosphorylation; **Plasmids**; Precipitin Tests; Protein Precursors--biosynthesis--BI; Protein Precursors--genetics--GE; Rubella Virus--metabolism--ME; Viral Structural Proteins--genetics--GE; Viral Structural Proteins--metabolism--ME

CAS Registry No.: 0 (Antibodies, Monoclonal); 0 (Plasmids); 0 (Protein Precursors); 0 (Viral Structural Proteins)

Record Date Created: 19910129

25/9/27 (Item 27 from file: 155)
 DIALOG(R) File 155:MEDLINE(R)

05832873 87033911 PMID: 3771631

Analysis of progressive deletions of the transmembrane and cytoplasmic domains of influenza hemagglutinin.

Doyle C; Sambrook J; Gething MJ

Journal of cell biology (UNITED STATES) Oct 1986, -103 (4) p1193-204, ISSN 0021-9525 Journal Code: HMV

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Subfile: INDEX MEDICUS

Site-directed oligonucleotide **mutagenesis** has been used to introduce chain **termination codons** into the cloned DNA sequences encoding the carboxy-terminal transmembrane (27 amino acids) and cytoplasmic (10 amino acids) domains of influenza virus hemagglutinin (HA). Four mutant genes

DIALOG

were constructed which express truncated forms of HA that lack the cytoplasmic domain and terminate at amino acids 9, 14, 17, or 27 of the wild-type hydrophobic domain. Analysis of the biosynthesis and intracellular transport of these mutants shows that the cytoplasmic tail is not needed for the efficient transport of HA to the cell surface; the stop-transfer sequences are located in the hydrophobic domain; 17 hydrophobic amino acids are sufficient to anchor HA stably in the membrane; and mutant proteins with truncated hydrophobic domains show drastic alterations in transport, membrane association, and stability.

Tags: Animal; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.

Descriptors: *Hemagglutinins, Viral--genetics--GE; Amino Acid Sequence; Base Sequence; Biological Transport; Cell Line; Cell Membrane--metabolism--ME; Cercopithecus aethiops; Chromosome Deletion; Fibroblasts--ultrastructure--UL; Hemagglutinin Glycoproteins, Influenza Virus; Hemagglutinins, Viral--metabolism--ME; Influenza A Virus, Human--analysis--AN; Kidney; **Recombinant** Proteins--genetics--GE; **Recombinant** Proteins--metabolism--ME

CAS Registry No.: 0 (Hemagglutinin Glycoproteins, Influenza Virus); 0 (Hemagglutinins, Viral); 0 (Recombinant Proteins)

Record Date Created: 19861126

?

25/3,AB/1 (Item 1 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

08924766 96251334 PMID: 8668201

Functional mapping of the translation-dependent instability element of yeast MATalpha1 mRNA.

Hennigan AN; Jacobson A
Department of Molecular Genetics and Microbiology, University of
Massachusetts Medical School, Worcester, 01655-0122, USA.
Molecular and cellular biology (UNITED STATES) Jul 1996, 16 (7)
p3833-43, ISSN 0270-7306 Journal Code: NGY
Contract/Grant No.: GM27757, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

The determinants of mRNA stability include specific cis-acting destabilizing sequences located within mRNA coding and noncoding regions. We have developed an approach for mapping coding-region instability sequences in unstable yeast mRNAs that exploits the link between mRNA translation and turnover and the dependence of nonsense-mediated mRNA decay on the activity of the UPF1 gene product. This approach, which involves the systematic insertion of in-frame translational **termination codons** into the coding sequence of a gene of interest in a upf1delta strain, differs significantly from conventional methods for mapping cis-acting elements in that it causes minimal perturbations to overall mRNA structure. Using the previously characterized MATalpha1 mRNA as a model, we have accurately localized its 65-nucleotide instability element (IE) within the protein coding region. Termination of translation 5' to this element stabilized the MATalpha1 mRNA two- to threefold relative to wild-type transcripts. Translation through the element was sufficient to restore an unstable decay phenotype, while internal termination resulted in different extents of mRNA stabilization dependent on the precise location of ribosome stalling. Detailed **mutagenesis** of the element's rare-codon/AU-rich sequence boundary revealed that the destabilizing activity of the MATalpha1 IE is observed when the terminal codon of the element's rare-codon interval is translated. This region of stability transition corresponds precisely to a MATalpha1 IE sequence previously shown to be complementary to 18S rRNA. Deletion of three nucleotides 3' to this sequence shifted the stability boundary one codon 5' to its wild-type location. Conversely, constructs containing an additional three nucleotides at this same location shifted the transition downstream by an equivalent sequence distance. Our results suggest a model in which the triggering of MATalpha1 mRNA destabilization results from establishment of an interaction between translating ribosomes and a downstream sequence element. Furthermore, our data provide direct molecular evidence for a relationship between mRNA turnover and mRNA translation.

25/3,AB/9 (Item 9 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07565576 92138613 PMID: 1735715

Mutagenesis of ribosomal protein S8 from Escherichia coli: defects in regulation of the spc operon.

Wower I; Kowaleski MP; Sears LE; Zimmermann RA
Department of Biochemistry and Molecular Biology, University of
Massachusetts, Amherst 01003.
Journal of Bacteriology (UNITED STATES) Feb 1992, 174 (4) p1213-21,
ISSN 0021-9193 Journal Code: HH3
Contract/Grant No.: GM-22807, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

The structural features of *Escherichia coli* ribosomal protein S8 that are involved in translational regulation of *spc* operon expression and, therefore, in its interaction with RNA have been investigated by use of a genetic approach. The *rpsH* gene, which encodes protein S8, was first inserted into an expression vector under the control of the *lac* promoter and subsequently **mutagenized** with methoxylamine or nitrous acid. A screening procedure based on the regulatory role of S8 was used to identify mutants that were potentially defective in their ability to associate with *spc* operon mRNA and, by inference, 16S mRNA. In this way, we isolated 39 variants of the S8 gene containing alterations at 34 different sites, including 37 that led to single amino acid substitutions and 2 that generated premature **termination codons**. As the mutations were distributed throughout the polypeptide chain, our results indicate that amino acid residues important for the structural integrity of the RNA-binding domain are not localized to a single segment. Nonetheless, the majority were located within three short sequences at the N terminus, middle, and C terminus that are phylogenetically conserved among all known eubacterial and chloroplast versions of this protein. We conclude that these sites encompass the main structural determinants required for the interaction of protein S8 with RNA.

25/3,AB/15 (Item 15 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

07015224 93019053 PMID: 1328486

Proteolytic processing of a Murray Valley encephalitis virus non-structural polyprotein segment containing the viral proteinase: accumulation of a NS3-4A precursor which requires mature NS3 for efficient processing.

Lobigs M

Division of Cell Biology, John Curtin School of Medical Research, Australian National University, Canberra.

Journal of general virology (ENGLAND) Sep 1992, 73 (Pt 9) p2305-12, ISSN 0022-1317 Journal Code: I9B

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The proteolytic processing of a non-structural polyprotein segment from the cytoplasmic domain of NS2A to the C terminus of NS5 of Murray Valley encephalitis (MVE) virus was examined, when expressed from cDNA via a vaccinia virus **recombinant**, in transiently transfected COS cells, or synthesized by cell-free translation. Cleavages mediated by the virus-encoded proteinase domain in NS3 at the junctions of NS2A-2B, NS2B-3 and NS4B-5 were catalysed efficiently. However, the cleavage at the NS3-4A junction, also mediated by the NS3 proteinase, was greatly delayed. Little or no NS3 was found, but an 85K precursor molecule accumulated; this was identified as NS3-4A. **Termination codons** were introduced by site-directed **mutagenesis** at the junctions of the NS3-4A, NS4A-4B and NS4B-5 genes to generate C-terminal truncations of the MVE virus polyprotein segment. In expression studies of these constructs the predicted NS3-mediated proteolytic cleavages were catalysed, except for that at the NS3-4A junction. In co-infections and co-transfections with constructs encoding the MVE virus nonstructural polyprotein region truncated at the C termini of NS3 or NS4A, efficient processing at the NS3-4A site was induced. Thus it appears that the MVE virus polyprotein is cleaved inefficiently in cis at the NS3-4A junction, whereas the site is processed efficiently in trans by mature NS3. The NS3-4A precursor is also seen in flavivirus-infected cells. Its function remains to be determined, but it could play a role in the replication of flavivirus, in view of the importance of polyprotein processing in the regulation of gene expression of positive-stranded RNA viruses, the modulation of processing at the NS3-4A site by NS3 or NS3-containing precursors described in the present

study and the importance of NS3 as an integral part of the viral polymerase complex.

25/3,AB/16 (Item 16 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06995914 92051377 PMID: 1719482

Rapid mapping by transposon mutagenesis of epitopes on the muscular dystrophy protein, dystrophin.

Sedgwick SG; Nguyen TM; Ellis JM; Crowne H; Morris GE
Genetics Division, MRC National Institute for Medical Research, Mill Hill, London, UK.

Nucleic acids research (ENGLAND) Nov 11 1991, 19 (21) p5889-94,
ISSN 0305-1048 Journal Code: O8L

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Antibody-binding epitopes in the central helical region of the muscular dystrophy protein, dystrophin, have been mapped using a new strategy of transposon **mutagenesis**. Tn1000 transposons carrying translation **termination codons** were introduced randomly by bacterial mating into a large fragment of dystrophin cDNA in a pEX2 **plasmid** to produce a library of transformants expressing truncated dystrophin fusion proteins. Epitopes were progressively lost as the expressed sequences were shortened, enabling the epitopes recognised by 22 monoclonal antibodies to be placed in order along the dystrophin molecule without in vitro manipulation of DNA. The C-terminus of each truncated fusion protein was precisely located within the dystrophin sequence by direct sequencing of pEX2 transformants using transposon-specific primers. Sequences as short as 7 and 17 amino-acids have been identified as essential for antibody binding in this way. Nineteen of the 22 monoclonal antibodies had been selected for their ability to bind both native and SDS-denatured dystrophin and 15 of these bind to one sequence of 74 amino-acids (residues 1431-1505 of the 3684 residue sequence). This may be an area of high immunogenicity or of close structural similarity between native dystrophin and the SDS-treated **recombinant** fragment used for immunization.

25/3,AB/17 (Item 17 from file: 155)
DIALOG(R) File 155:MEDLINE(R)

06986006 90316123 PMID: 2142454

Cleavage-site preferences of Sindbis virus polyproteins containing the non-structural proteinase. Evidence for temporal regulation of polyprotein processing in vivo.

de Groot RJ; Hardy WR; Shirako Y; Strauss JH
Division of Biology, California Institute of Technology, Pasadena 91125.
EMBO journal (ENGLAND) Aug 1990, 9 (8) p2631-8, ISSN 0261-4189
Journal Code: EMB

Contract/Grant No.: AI10793, AI, NIAID; AI20612, AI, NIAID

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The non-structural proteins of Sindbis virus, nsP1, 2, 3 and 4, are produced upon cleavage of polyproteins P123 and P1234 by a proteinase residing in nsP2. We used cell free translation of SP6 transcripts to study the proteolytic activity of nsP2 and of nsP2-containing polyproteins. To generate polyprotein enzymes, a set of **plasmids** was made in which cleavage sites were eliminated and new initiation and **termination codons** introduced by in vitro **mutagenesis**. As a substrate, we used a polyprotein in which the nsP2 proteinase had been inactivated by a single amino acid substitution. All nsP2-containing polyproteins cleaved the

nsP1/2 site in trans. However, proteinases containing nsP1 were unable to cleave the nsP2/3 site. Furthermore, only proteinases containing nsP3 could cleave the nsP3/4 site. These differences in cleavage site specificity result in a temporal regulation of processing in vivo. At 1.7 h post infection P123 and nsP4 accumulated and only small amounts of P34 were found. However, at 4 h post infection P123 was processed rapidly and P34 was produced rather than nsP4. Since nsP4 is thought to be the viral RNA polymerase, the temporal regulation of the nsP4/P34 ratio may be responsible for the temporal regulation of RNA synthesis.

25/3,AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06955578 90245560 PMID: 2186364

Programmed ribosomal frameshifting generates the Escherichia coli DNA polymerase III gamma subunit from within the tau subunit reading frame.

Blinkowa AL; Walker JR

Department of Microbiology, University of Texas, Austin 78712.

Nucleic acids research (ENGLAND) Apr 11 1990, 18 (7) p1725-9, ISSN 0305-1048 Journal Code: O8L

Contract/Grant No.: GM34471, GM, NIGMS

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The Escherichia coli dnaX gene encodes both the tau and gamma subunits of DNA polymerase III holoenzyme in one reading frame. The 71.1 kDa tau and the shorter gamma share N-terminal sequences. **Mutagenesis** of a potential ribosomal frameshift signal located at codons 428-430 without changing the amino acid sequence of the tau product, eliminated detectable synthesis of the gamma subunit, suggesting that the reading frame is shifted at that sequence and gamma is terminated by a nonsense codon located in the -1 frame 3 nucleotides downstream of the signal. This seems to be the first known case of a frameshift which is used, along with the **termination codon** in the -1 frame, to terminate a peptide within a reading frame. [**Mutagenesis** of a dibasic peptide (lys-lys) at codons 498-499, the site at which a tau'-LacZ fusion protein was cleaved in vitro (1) had no effect on gamma formation in vivo, suggesting that cleavage observed in vitro is not the mechanism of gamma formation in vivo.
?

Set	Items	Description
S1	739	AU="MURPHY B"
S2	543	AU="MURPHY B R"
S3	161	AU="MURPHY B."
S4	333	AU="MURPHY B.R."
S5	44	AU="MURPHY BRIAN"
S6	191	AU="MURPHY BRIAN R" OR AU="MURPHY BRIAN ROBERT" OR AU="MURPHY BRIAN ROBERTS"
S7	1921	AU="COLLINS P"
S8	206	AU="COLLINS P L"
S9	414	AU="COLLINS P."
S10	209	AU="COLLINS P.L."
S11	138	AU="COLLINS PETER" OR AU="COLLINS PETER AIRDRI LIMITED TECHNOLOGY HOUSE"
S12	114	AU="COLLINS PETER L"
S13	137	AU="WHITEHEAD S"
S14	52	AU="WHITEHEAD S S" OR AU="WHITEHEAD S."
S15	47	AU="WHITEHEAD S.S."
S16	48	AU="WHITEHEAD STEPHEN" OR AU="WHITEHEAD STEPHEN S"
S17	65	AU="BUKREYEV A" OR AU="BUKREYEV A A" OR AU="BUKREYEV A." OR AU="BUKREYEV A.A." OR AU="BUKREYEV AA" OR AU="BUKREYEV ALEXANDER" OR AU="BUKREYEV ALEXANDER A"
S18	1	AU="JAHASZ, K."
S19	221	AU="TENG M" OR AU="TENG M N" OR AU="TENG M."
S20	21	AU="TENG M.N."
S21	17	AU="TENG MICHAEL" OR AU="TENG MICHAEL N"
S22	8447523	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 - OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21
S23	29770	RESPIRATORY(W) SYNCYTIAL(W) VIRUS
S24	26911	RSV
S25	45480	S23 OR S24
S26	11376	S22 AND S25
S27	10	S14 AND S25
S28	5242	S1 OR S2 OR S3 OR S4 OR S5 OR S6 OR S7 OR S8 OR S9 OR S10 - OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 OR S20 OR S21
S29	557	S25 AND S28
S30	556886	VACCINE? ? OR IMMUNOGENIC
S31	1852222	RECOMBINANT? OR VECTOR? ?
S32	288	S29 AND S30
S33	1623964	TRANSLAT?
S34	1325445	TERMINAT?
S35	172324	CODON
S36	175	S32 AND S31
S37	426108	ATTENUATE? ?
S38	21841	MATERNAL(S) ANTIBOD?
S39	248	S29 AND S31
S40	175	S32 AND S39
S41	103	RD (unique items)
S42	48	S41 NOT PY>1996
S43	180	S29 AND S30 AND S37
S44	95	RD (unique items)
S45	24	S44 NOT PY>1996
S46	63	S42 OR S45
S47	19003	S33(S)S34(S)S35
S48	1	S46 AND S47
S49	5	S38 AND S46
S50	5	RD (unique items)
S51	10093	S25 AND S30

DIALOG

S52	1570	S47 AND S51
S53	1568	S31 AND S52
S54	4835	S33(3N) S34(3N) S35
S55	603	S53 AND S54
S56	603	RD (unique items)
S57	603	S30 AND S56
S58	18829	S25/TI
S59	11	S57 AND S58
S60	7	S59 NOT PY>1996
?		

2, August 14, 2001, 16:14

Copied from 09444422 on 26-02-2004

PATENT NO.: 5,149,650
ISSUED: September 22, 1992 (19920922)
INVENTOR(s): Wertz, Gail W., Birmingham, AL (Alabama), US (United States of America)
Collins, Peter L., Rockville, MD (Maryland), US (United States of America)
ASSIGNEE(s): University of North Carolina at Chapel Hill, (A U.S. Company or Corporation), Chapel Hill, NC (North Carolina), US (United States of America)
[Assignee Code(s): 5583]
EXTRA INFO: Assignment transaction [Reassigned], recorded August 28, 1998 (19980828)
APPL. NO.: 7-218,737
FILED: July 13, 1988 (19880713)

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation in part of International Patent Application Number PCT-US86-02756, filed 23 Dec. 1986, which is a continuation-in-part of U.S. patent application Ser. No. 818,740, filed 14 Jan. 1986, abandoned.

FULL TEXT: 1438 lines

ABSTRACT

This invention discloses compositions of DNA and proteins that are useful for preparing **vaccines** against human **respiratory syncytial virus** [HRSV]. The DNA compositions include structural genes coding for native structural viral proteins and **immunogenic** fragments of these proteins. Host cells transformed with the above DNA compositions are also disclosed. **Vaccines** made from the native structural viral proteins or **immunogenic** fragments are also disclosed as well as methods for protecting humans by inoculation with these **vaccines** .
?

50/3,AB/1 (Item 1 from file: 5)
 DIALOG(R) File 5:Biosis Previews(R)
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09997450 BIOSIS NO.: 199598452368

Cold-passaged, temperature-sensitive mutants of human respiratory syncytial virus (RSV) are highly attenuated, immunogenic, and protective in seronegative chimpanzees, even when RSV antibodies are infused shortly before immunization.

AUTHOR: Crowe James E Jr(a); Bui Phuong T; Siber George R; Elkins William R
 ; Chanock Robert M; **Murphy Brian R**

AUTHOR ADDRESS: (a)Respiratory Viruses Sect., Lab. Infectious Diseases,
 Natl. Inst. Allergy Infectious Diseases, Na**USA

JOURNAL: Vaccine 13 (9):p847-855 1995

ISSN: 0264-410X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A cold-passaged (cp) temperature-sensitive (ts) **RSV** mutant, designated **RSV** cpts-530, which possesses host-range mutations acquired during 52 passages at low temperature in bovine tissue culture and one or more ts mutations induced by chemical mutagenesis (shut-off temperature 39 degree C) was found previously to be tenfold restricted in its replication in mice as compared to wild-type virus and stable genetically in nude mice. In the current study, we introduced additional attenuating mutations, such as small-plaque (sp) or ts mutations, into cpts-530 by chemical mutagenesis with 5-fluorouracil, with the intent of obtaining derivatives of cpts-530 that were more **attenuated** in mice or chimpanzees and that were more stable genetically following replication in vivo. Fourteen mutants of **RSV** cpts-530 which had acquired an additional ts mutation were identified and found to be more restricted in replication in BALB/c mice than the cpts-530 parental strain. One mutant, designated cpts-530/1009 (shut-off temperature 36 degree C), was 30 times more restricted in replication in the nasal turbinates of mice and threefold more restricted in the nasopharynx of seronegative chimpanzees than its cpts-530 parent. Like its parent, this mutant was highly restricted (30 000-fold) in replication in the lower respiratory tract of chimpanzees even following direct intratracheal inoculation. The cpts-530 and cpts-530/1009 mutants exhibited a high level of stability of the ts phenotype during replication in chimpanzees. The immunogenicity and protective efficacy of the cpts-530/1009 mutant and that of two other previously described candidate live **attenuated RSV vaccines** were compared in seronegative chimpanzees, some of whom were pretreated with **RSV** immune globulin by the intravenous route to simulate the condition of the very young infant who possesses passively acquired **maternal antibodies**. The three candidate **vaccine** strains were **immunogenic** and induced significant resistance to **RSV** challenge in both groups of chimpanzees. Interestingly, the chimpanzees infused with **RSV antibodies** prior to immunization were primed more effectively for an unusually high serum neutralizing **antibody** response to infection with challenge virus than chimpanzees which did not receive such **antibodies**. This high level booster response occurred despite marked restriction of replication of the challenge virus. Thus, the cpts-530/1009 virus and related mutants exhibit many desirable characteristics which make them promising **vaccine** candidates.

1995

1, August 14, 2001, 12:31

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50/3,AB/2 (Item 2 from file: 5)
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09230715 BIOSIS NO.: 199497239085

An update on approaches to the development of respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) vaccines.

AUTHOR: **Murphy Brian R** (a); Hall Susan L; Kulkarni Arun B; Crowe James E Jr; **Collins Peter L**; Connors Mark; Karron Ruth A; Chanock Robert M

AUTHOR ADDRESS: (a)Lab. Infect. Dis., Natl. Inst. Allergy Infect. Dis., Natl. Inst. Health, Bethesda, MD**USA

JOURNAL: Virus Research 32 (1):p13-36 1994

ISSN: 0168-1702

DOCUMENT TYPE: Literature Review

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: RSV and PIV3 are responsible for about 30% of severe viral respiratory tract disease leading to hospitalization of infants and children. For this reason, there is a need to develop **vaccines** effective against these viruses. Since these viruses cause severe disease in early infancy, **vaccines** must be effective in the presence of **maternal antibody**. Currently, several strategies for immunization against these viruses are being explored including peptide **vaccines**, subunit **vaccines**, vectored **vaccines** (e.g., vaccinia-RSV or adenovirus-RSV **recombinants**), and live **attenuated virus vaccines**. The current status of these approaches is reviewed. In addition, the immunologic basis for the disease potentiation seen in vaccinees immunized with formalin-inactivated RSV during subsequent RSV infection is reviewed. The efficacy of immunization in the presence of **maternal antibody** is discussed. Much progress for a RSV and PIV3 **vaccine** has been made and successful immunization against each of these pathogens should be achieved within this decade.

1994

50/3,AB/3 (Item 1 from file: 348)
 DIALOG(R)File 348:EUROPEAN PATENTS
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00278345

VACCINES FOR HUMAN RESPIRATORY VIRUS.

IMPfstoffe gegen menschliche respiratorische Viren.

VACCINS CONTRE LE VIRUS RESPIRATOIRE HUMAIN.

PATENT ASSIGNEE:

UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL, (751080), , Chapel Hill, North Carolina 27514, (US), (applicant designated states: AT;BE;CH;DE;FR;GB;IT;LI;LU;NL;SE)

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PATENT (CC, No, Kind, Date): EP 290446 A1 881117 (Basic)

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PRIORITY (CC, No, Date): US 818740 860114

DESIGNATED STATES: AT; BE; CH; DE; FR; GB; IT; LI; LU; NL; SE

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INTERNATIONAL PATENT CLASS: C12N-015/45; A61K-039/155;

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Total word count - documents A + B			5222

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DIALOG(R)File 349:PCT Fulltext

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00226847

VACCINES FOR HUMAN RESPIRATORY VIRUS

VACCINS CONTRE LE VIRUS RESPIRATOIRE HUMAIN

Patent Applicant/Assignee:

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Fulltext Word Count: 15772

English Abstract

Compositions of DNA and protein that are useful for preparing **vaccines** against human **respiratory syncytial virus** (HRSV). The proteins include the native structural viral proteins and **immunogenic** fragments thereof. The DNA compositions include structural genes coding for these proteins and expression and replication plasmids containing the structural genes. Host cells transformed with the above DNA compositions are also disclosed herein. Lastly **vaccines** comprised of the native structural viral proteins and their **immunogenic** derivatives are disclosed as well as methods for protecting humans by inoculation with said **vaccines**.

Japanese Abstract

Compositions d'ADN et de proteines permettant de preparer des vaccins contre le virus humain respiratoire syncytial (HRSV). Les proteines comprennent des proteines virales structurales originales et des fragments immunogenes de celles-ci. Les compositions d'ADN comprennent des genes structuraux codant lesdites proteines et des plasmides d'expression et de replication contenant les genes structuraux. Sont egalement decrites des cellules hotes transformees avec lesdites compositions d'ADN. Sont egalement decrits des vaccins comprenant les proteines virales structurales originales et leurs derives immunogenes ainsi que des procedes de protection des etres humains par inoculation desdits vaccins.

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46/3,AB/2 (Item 1 from file: 5)
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10802105 BIOSIS NO.: 199799423250

Acquisition of the ts phenotype by a chemically mutagenized cold-passaged human respiratory syncytial virus vaccine candidate results from the acquisition of a single mutation in the polymerase (L) gene.

AUTHOR: Crowe James E Jr; Firestone Cai-Yen; Whitehead Stephen S ;

Collins Peter L ; Murphy Brian R

AUTHOR ADDRESS: Respiratory Viruses Section, Lab. Infectious Diseases,
 National Inst. Allergy Infectious Diseases, N**USA

JOURNAL: Virus Genes 13 (3):p269-273 1996

ISSN: 0920-8569

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: A cold-passaged (cp) temperature-sensitive (ts) mutant of human **respiratory syncytial virus** designated **RSV** cpts-248 was previously derived by random chemical mutagenesis of the non-ts mutant cp-**RSV** that possesses one or more host range mutations. We previously demonstrated in rodents and seronegative chimpanzees that the cpts-248 virus is more **attenuated** than cp-**RSV** and is more stable genetically than previously isolated **RSV** ts mutants. In the present study, we determined that the acquisition of the ts phenotype and the increased attenuation of the cpts-248 virus are associated with a single nucleotide substitution at nucleotide 10,989 that results in a change in the coding region (amino acid position 831) of the polymerase gene. The identification of this attenuating ts mutation is important because cpts-248 was used as the parent virus for the generation of a number of further **attenuated** mutants that are currently being evaluated as candidate **vaccine** strains in clinical trials in infants. Furthermore, technology now exists to rationally design new **vaccine** candidates by incorporating multiple attenuating mutations, such as the one identified here, into infectious viruses that are genetically stable and appropriately **attenuated**.

1996

46/3,AB/3 (Item 2 from file: 5)
 DIALOG(R)File 5:Biosis Previews(R)
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10696789 BIOSIS NO.: 199799317934

Nucleotide sequence analysis of the respiratory syncytial virus subgroup A cold-passaged (cp) temperature sensitive (ts) cpts-248/404 live attenuated virus vaccine candidate.

AUTHOR: Firestone Cai-Yen; Whitehead Stephen S ; Collins Peter L ;

Murphy Brian R (a); Crowe James E Jr

AUTHOR ADDRESS: (a)Respiratory Viruses Sect., Lab. Infect. Dis., Natl.
 Inst. Allergy Infect. Dis., Natl. Inst. Heal**USA

JOURNAL: Virology 225 (2):p419-422 1996

ISSN: 0042-6822

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: The complete nucleotide sequence of the **RSV** cpts-248/404 live **attenuated vaccine** candidate was determined from cloned cDNA and was compared to that of the **RSV** A2/HEK7 wild-type, cold-passaged cp-**RSV**, and cpts-248 virus, which constitute the series of progenitor viruses.

RSV cpts-248/404 is more **attenuated** and more temperature sensitive (ts) (shutoff temperature 36 degree) than its cpts-248 parent virus (shut-off temperature 38 degree) and is currently being evaluated in phase I clinical trials in humans. Our ultimate goal is to identify the genetic basis for the host range attenuation phenotype exhibited by cp-**RSV** (i.e., efficient replication in tissue culture but decreased replication in chimpanzees and-humans) and for the ts and attenuation phenotypes of its chemically mutagenized derivatives, cpts-248 and cpts-248/404. Compared with its cpts-248 parent, the cpts-248/404 virus possesses an amino acid change in the polymerase (L) protein and a single nucleotide substitution in the M2 gene start sequence. In total, the cpts-248/404 mutant differs from its wild-type **RSV** A2/HEK7 progenitor in seven amino acids (four in the polymerase (L) protein, two in the fusion (F) glycoprotein, and one in the (N) nucleoprotein) and one nucleotide difference in the M2 gene start sequence. Heterogeneity at nucleotide position 4 (G or C, negative sense, compared to G in the **RSV** A2/HEK7 progenitor) in the leader region of vRNA developed during passage of the cpts-248/404 in tissue culture. Biologically cloned derivatives of **RSV** cpts-248/404 virus that differed at position 4 possessed the same level of temperature sensitivity and exhibited the same level of replication in the upper and lower respiratory tract of mice, suggesting that heterogeneity at this position is not clinically relevant. The determination of the nucleotide sequence of the cpts-248/404 virus will allow evaluation of the stability of the eight mutations that are associated with the attenuation phenotype during **vaccine** production and following replication in humans.

1996

46/3,AB/4 (Item 3 from file: 5)
 DIALOG(R)File 5:Biosis Previews(R)
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10369534 BIOSIS NO.: 199698824452

Live subgroup B respiratory syncytial virus vaccines that are attenuated, genetically stable, and immunogenic in rodents and nonhuman primates.

AUTHOR: Crowe James E Jr(a); Bui Phuong T; Firestone Cai-Yen; Connors Mark; Elkins William R; Chanock Robert M; **Murphy Brian R**

AUTHOR ADDRESS: (a)D-7235 MCN, VUMC, Nashville, TN 37232-2581**USA

JOURNAL: Journal of Infectious Diseases 173 (4):p829-839 1996

ISSN: 0022-1899

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Optimal immunization of neonates against disease caused by **respiratory syncytial virus (RSV)** probably will require multiple doses of a **vaccine** containing viruses of both subgroups A and B. Live subgroup B **RSV** mutants were generated containing multiple attenuating mutations, ts (temperature-sensitive) and non-ts (host range), that were introduced by prolonged passage in cell culture or by chemical mutagenesis. The cold-passaged (cp)-52 mutant was restricted in replication compared to wild type virus in rodents and nonhuman primates. In addition, the attenuation phenotype of cp-52 was stable after prolonged replication in immunosuppressed rodents. One or two ts mutations were then introduced into the cp-52 mutant to generate additional candidate **vaccine** strains that were more **attenuated** in vivo than the cp-52 parental virus. Tests in humans are being done to determine if one or more of the **RSV** B-1 mutants exhibit a satisfactory

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balance between attenuation and immunogenicity.

1996

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10178274 BIOSIS NO.: 199698633192

Production of infectious human respiratory syncytial virus from cloned cDNA confirms an essential role for the transcription elongation factor from the 5' proximal open reading frame of the M2 mRNA in gene expression and provides a capability for vaccine development.

AUTHOR: Collins Peter L (a); Hill Myron G; Camargo Ena; Grosfeld Haim; Chanock Robert M; Murphy Brian R

AUTHOR ADDRESS: (a)Lab. Infectious Dis., 7 Center Drive, MS 0720, Natl. Inst. Allergy Infectious Dis., Bethesda, MD**USA

JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 92 (25):p11563-11567 1995

ISSN: 0027-8424

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Infectious human **respiratory syncytial virus (RSV)** was produced by the intracellular coexpression of five plasmid-borne cDNAs. One cDNA encoded a complete positive-sense version of the **RSV** genome (corresponding to the replicative intermediate RNA or antigenome), and each of the other four encoded a separate **RSV** protein, namely, the major nucleocapsid N protein, the nucleocapsid P phosphoprotein, the major polymerase L protein, or the protein from the 5' proximal open reading frame of the M2 mRNA (M2(ORF1)). **RSV** was not produced if any of the five plasmids was omitted. The requirement for the M2(ORF1) protein is consistent with its recent identification as a transcription elongation factor and confirms its importance for **RSV** gene expression. It should thus be possible to introduce defined changes into infectious **RSV**. This should be useful for basic studies of **RSV** molecular biology and pathogenesis; in addition, there are immediate applications to the development of live **attenuated vaccine** strains bearing predetermined defined attenuating mutations.

1995

46/3,AB/6 (Item 5 from file: 5)
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09997450 BIOSIS NO.: 199598452368

Cold-passaged, temperature-sensitive mutants of human respiratory syncytial virus (RSV) are highly attenuated, immunogenic, and protective in seronegative chimpanzees, even when RSV antibodies are infused shortly before immunization.

AUTHOR: Crowe James E Jr(a); Bui Phuong T; Siber George R; Elkins William R; Chanock Robert M; Murphy Brian R

AUTHOR ADDRESS: (a)Respiratory Viruses Sect., Lab. Infectious Diseases, Natl. Inst. Allergy Infectious Diseases, Na**USA

JOURNAL: Vaccine 13 (9):p847-855 1995

ISSN: 0264-410X

DOCUMENT TYPE: Article

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RECORD TYPE: Abstract
 LANGUAGE: English

ABSTRACT: A cold-passaged (cp) temperature-sensitive (ts) **RSV** mutant, designated **RSV** cpts-530, which possesses host-range mutations acquired during 52 passages at low temperature in bovine tissue culture and one or more ts mutations induced by chemical mutagenesis (shut-off temperature 39 degree C) was found previously to be tenfold restricted in its replication in mice as compared to wild-type virus and stable genetically in nude mice. In the current study, we introduced additional attenuating mutations, such as small-plaque (sp) or ts mutations, into cpts-530 by chemical mutagenesis with 5-fluorouracil, with the intent of obtaining derivatives of cpts-530 that were more **attenuated** in mice or chimpanzees and that were more stable genetically following replication in vivo. Fourteen mutants of **RSV** cpts-530 which had acquired an additional ts mutation were identified and found to be more restricted in replication in BALB/c mice than the cpts-530 parental strain. One mutant, designated cpts-530/1009 (shut-off temperature 36 degree C), was 30 times more restricted in replication in the nasal turbinates of mice and threefold more restricted in the nasopharynx of seronegative chimpanzees than its cpts-530 parent. Like its parent, this mutant was highly restricted (30 000-fold) in replication in the lower respiratory tract of chimpanzees even following direct intratracheal inoculation. The cpts-530 and cpts-530/1009 mutants exhibited a high level of stability of the ts phenotype during replication in chimpanzees. The immunogenicity and protective efficacy of the cpts-530/1009 mutant and that of two other previously described candidate live **attenuated RSV vaccines** were compared in seronegative chimpanzees, some of whom were pretreated with **RSV** immune globulin by the intravenous route to simulate the condition of the very young infant who possesses passively acquired maternal antibodies. The three candidate **vaccine** strains were **immunogenic** and induced significant resistance to **RSV** challenge in both groups of chimpanzees. Interestingly, the chimpanzees infused with **RSV** antibodies prior to immunization were primed more effectively for an unusually high serum neutralizing antibody response to infection with challenge virus than chimpanzees which did not receive such antibodies. This high level booster response occurred despite marked restriction of replication of the challenge virus. Thus, the cpts-530/1009 virus and related mutants exhibit many desirable characteristics which make them promising **vaccine** candidates.

1995

46/3,AB/7 (Item 6 from file: 5)
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09872880 BIOSIS NO.: 199598327798

A cold-passaged attenuated strain of human respiratory syncytial virus contains mutations in the F and L genes.

AUTHOR: Connors Mark; Crowe James E Jr; Firestone Cai-Yen; **Murphy Brian R**
 ; **Collins Peter L** (a

AUTHOR ADDRESS: (a)Respiratory Viruses Sect., Lab. Infect. Dis., Natl.
 Inst. Allergy Infect. Dis., NIH, 9000 Rockvi**USA

JOURNAL: Virology 208 (2):p478-484 1995

ISSN: 0042-6822

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

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ABSTRACT: In previous studies, a mutant (cp-**RSV**) of the **RSV** A2 strain derived from 52 serial cold passages in bovine embryonic tissue culture was highly **attenuated** in seropositive adults and children but caused upper respiratory tract disease in seronegative infants. We investigated the genetic basis for this attenuation phenotype by comparing the complete genomic RNA sequence of this virus with the published sequence of strain A2 as well as with that of its unattenuated wild-type parent (HEK-7) virus. RNA was extracted from virions grown in tissue culture, reverse transcribed, amplified by the polymerase chain reaction (PCR), cloned, and sequenced. Changes from the published A2 wild-type sequence were confirmed on independently derived cDNA clones and by direct sequencing of PCR fragments. The HEK-7 parent virus was then analyzed at these positions by direct sequencing of PCR fragments. Fifteen nucleotide differences between the published A2 wild-type virus and cp-**RSV** were found. None appeared to involve cis-acting RNA sequences. Of the 15 nucleotide differences, only 1 occurred outside a translational open reading frame (ORF), and 2 which did occur within ORFs were silent at the amino acid level. The remaining 12 nucleotide differences encoded amino acid changes. All 3 of the mutations which were silent at the amino acid level, and 8 of the 12 which resulted in amino acid differences, were also present in the HEK-7 parent virus and therefore were not changes acquired during the cold passages. Thus, the remaining 4 nucleotide differences and the attendant 4 amino acid changes are associated with the attenuation phenotype of the cp-**RSV**. Two of the changes occur in the F gene and two in the L gene. These results confirm the previously described **RSV** genomic sequence, provide the first sequence of a live **attenuated RSV vaccine** strain, provide the first sequence of an **RSV** strain which has been evaluated in chimpanzees and humans, and indicate that attenuation in humans of a pneumovirus can be associated with a relatively small number of nucleotide and amino acid changes.

1995

46/3,AB/8 (Item 7 from file: 5)
 DIALOG(R) File 5: Biosis Previews(R)
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09831324 BIOSIS NO.: 199598286242

Isolation and characterization of a highly attenuated respiratory syncytial virus (RSV) vaccine candidate by mutagenesis of the incompletely attenuated RSV A2 ts-1 NG-1 mutant virus.

AUTHOR: Hsu Kuo-Hom L(a); Crowe James E Jr; Lubeck Michael D; Davis Alan R; Hung Paul P; Chanock Robert M; **Murphy Brian R**

AUTHOR ADDRESS: (a)145/R-2, Wyeth-Ayerst Research, P.O. Box 8299, Philadelphia, PA 19101**USA

JOURNAL: Vaccine 13 (5):p509-515 1995

ISSN: 0264-410X

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Ts-1, a temperature sensitive (ts) mutant of **RSV**, was previously derived from **RSV** A2 virus by mutagenesis with 5-fluorouracil (5-FU). Ts-1 was **attenuated** for adult volunteers and seropositive children but retained a low level of virulence in seronegative infant vaccinees as indicated by the occurrence of upper respiratory tract disease. Ts-1 NG-1, a more defective derivative of ts-1, was produced by mutagenesis of ts-1 with nitrosoguanidine. However, ts-1 NG-1 still retained a low level of virulence for the upper respiratory tract and showed some genetic instability in chimpanzees. With renewed interest in

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